Preliminary report on taxonomy of

Erigeron lackschewitzii

for

Montana Natural Heritage Program

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This is an abridged report

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ABSTRACT

Preliminary data obtained from chloroplast DNA and morphological analyses suggest that *Erigeron lackschewitzii* is an apomictic race of the predominantly sexual species, *E. ochroleucus*. Specifically the hypothesis that outcrossing races of *E. ochroleucus* bud off apomictic races, one of which is *E. lackschewitzii*, and another possibly *E. radicatus*, will be evaluated with more indepth genetic and morphological analyses. Furthermore, the potential inclusion into synonymy of *E. radicatus* with *E. ochroleucus* var. *scribneri* will be evaluated.

INTRODUCTION

Nesom and Weber (1983) proposed a new alpine species, *Erigeron lackschewitzii*, endemic to an area largely within the Bob Marshall and Scapegoat Wildernesses of northwestern Montana. The authors suggested that *E. lackschewitzii* may be derived from either *E. simplex* Greene, a widespread sympatric species, or the southern alpine race of *E. grandiflorus* Hook., a species distributed from the Beartooth Plateau south into New Mexico and west into the Great Basin (Spongberg, 1972). Dorn (1984 and personal communication) suggests *E. lackschewitzii* is synonymous with *E. ochroleucus* Nutt., specifically *E. ochroleucus* var. *scribneri* (Canby) Cronq.

This preliminary report addresses the taxonomic status of *E. lackschewitzii* and represents the initial step in a comprehensive study of the taxonomy of this putative species for the Montana Natural Heritage Program. The report focuses on comparative morphological and chloroplast DNA (cpDNA) studies, chromosome counts, and SEM microscopy to elucidate the relationship of *E. lackschewitzii* to *E. simplex* , *E. ochroleucus* and other putative close relatives.

Erigeron radicatus Hook. has been included in this paper in response to my preliminary findings which suggest that this species is closely related to, and may be a variety of, *E. ochroleucus*. Arthur Cronquist's personal communication to Klaus Lackschewitz questioned the taxonomic distinction of *E. radicatus* (Lackschewitz, personal communication), while Peter Lesica intimates that *E. radicatus* may be indistinguishable morphologically from *E. ochroleucus* var. *scribneri* (Lesica, personal communication).

METHODS AND MATERIALS

Specimens for cpDNA screening were obtained from eight sites in western Montana (Table 1). Whole plants were either pressed and dried or placed in plastic bags on ice and later stored in a -80°C freezer.

Leaves were ground in CTAB DNA extraction buffer (Doyle et al., 1990). Total DNA from nine specimens representing six species was digested with 12 restriction endonucleases having six-base nucleotide recognition sequences. These included *Bam*HI, *Bcl*I, *Dra*I, *Asc*I, *Sac*I, *Xho*I, *Eco*RI, *Eco*RV, *Nsi*I, *Sca*I, *Stu*I, and *Xba*I. Fragments were separated in 0.9% agarose/TBE gels. *Erigeron lackschewitzii*, *E. ochroleucus*, and *E. radicatus*, as the ingroup, were compared against the outgroup *E. simplex*, *E. speciosus*, and *E. caespitosus* in order to polarize site mutation.

Restriction site analysis, including Southern blotting, nick-translation, hybridization to radioactive probes, and autoradiography basically follow Sytsma and Gottlieb (1986). Cloned fragments from the large single copy region of the cpDNA library of *Petunia* (provided by Jeff Palmer) and from the nuclear ribosomal repeat of *Glycine max* (PGMR1, provided by Liz Zimmer) were used in the hybridization experiments. Figure 1 illustrates location of cpDNA probe regions.

Morphological analyses were based on 30 quantitative and 27 qualitative characters. These attributes were scored for each of 73 individual plants representing four species: *E. lackschewitzii* (34 specimens), *E. ochroleucus* var. *scribneri* (9 specimens), *E. radicatus* (11 specimens), and

E. *simplex* (20 specimens). See Table 2 for itemization of characters. Specimens were collected July 17 to August 5, 1992, from the Bob Marshall Wilderness, Lewis and Clark National Forest, northwestern Montana, and the Bridger Range, Gallatin National Forest, in southwestern Montana. Phenetic analysis, including calculation of a phenetic distance matrix, cluster analysis, and ordination, was performed using the computer program NTSYS (Applied Biostatistics, Inc.).

Chromosome counts for *E. lackschewitzii* were attempted using standard anther squash and staining techniques. Seed germination was attempted according to procedures outlined by Spongberg (1972) on four dried mature heads by placing disk and ligule achenes on moist filter paper in a cool refrigerator at 25°C for one week to simulate stratification prior to germination.

Specimens of *E. simplex*, *E. ochroleucus*, *E. radicatus*, and *E. lackschewitzii* were sent to Western State College, Gunnison, Colorado, for scanning electron microscopic analysis of pollen grains upon request from Wayne Warnken as a student project.

RESULTS

DNA restriction site variation

Of the twelve restriction endonucleases, informative fragment length variations were found with four (Table 3), while the remaining eight are still under study. Chloroplast DNA probe regions S6 and P3 displayed greatest variation with five mutations each; probe regions S8 and P8 each had two mutations, while no significant variation was found in probe region P6. Unique cpDNA fragment sizes were found for five of the six species.

Regarding nuclear rDNA repeat regions, *Bcl*I revealed a site responsible for a possible 7.8kb doublet specific to *E. radicatus*, one *E. simplex* (ES13), *E. speciosus* and *E. caespitosus*, and another site responsible for 3.4kb + 7.2kb fragments potentially unique to the second *E. simplex* (ES21). Other nuclear rDNA variation exists and is in the process of being analyzed.

A dendrogram based on the presence of shared restriction sites revealed greater distances between the ingroup and outgroup than within each group (Figure 2). Specifically, *E. lackschewitzii* is most similar genetically to *E. radicatus* and *E. ochroleucus*, based on this sample. These distances will be converted to genetic distances in the final report.

Morphological studies

Ordination of the phenetic data set revealed a distinct clustering of individuals belonging to *E. simplex* and an indistinct clustering of individuals belonging to *E. ochroleucus*, *E. lackschewitzii*, and *E. radicatus* (Figures 3 and 4). Figure 5 illustrates the cluster analysis of phenetic distances calculated between all pairwise comparisons of individuals of *E. simplex*, *E. radicatus*, *E. ochroleucus* and *E. lackschewitzii*. *Erigeron lackschewitzii* specimen EL203 was the only individual to be placed a relatively great phenetic distance from the other *E. lackschewitzii* specimens due to its unusual robustness and multicipital flowering stem. But, clearly, the large marjority of individuals of *E. lackschewitzii* are morphologically most similar to each other or to *E. ochroleucus*.

Chromosome count and pollen SEM

Chromosome numbers for *E. lackschewitzii* were unobtainable; no pollen was found in any of the capitula examined. Seed germination was likewise unsuccessful, as achenes were found to be too immature to germinate.

A single pollen grain of *E. lackschewitzii* was photomicrographed and found to be abnormal (Figure 6), while pollen grains of *E. simplex* and *E. ochroleucus* were typical (Figures 7 and 8) of the Astereae tribe of Asteraceae (Skvarla, et al., 1977). *E. radicatus* pollen has not been photomicrographed to date.

DISCUSSION

Preliminary cpDNA and phenetic analysis both strongly suggest that *E. lackschewitzii* is intimately related to *E. ochroleucus* and *E. radicatus* and differs clearly from the outroup complex of *E. simplex*, *E. speciosus*, and *E. caespitosus*. Individuals within each group also displayed unique sequences which will require further study. Additional DNA from other specimens will be isolated to adequately determine whether initial results continue to display consistent differences as outgroups are changed.

Preliminary DNA restriction site variations and morphological data call into doubt the species status of *E. lackschewitzii*. Nesom (1989 and personal communication) and Dorn (1984 and personal communication) have suggested that *E. lackschewitzii* does not warrant specific status. However, the taxonomic status of *E. lackschewitzii* still needs to be investigated. For example, Huber and Leuchtmann (1992) invoke recent speciation during glaciation epochs to account for genetic uniformity of nine alpine *Erigerons* in the Alps. It could be that *E. lackschewitzii* belongs to a species complex that has only recently evolved and does not show much divergence genetically. This hypothesis will be evaluated with allozyme studies of all putative relatives of *E. lackschewitzii* during the second year of my study.

Morphological data is very preliminary for three reasons. Firstly, very few *E. ochroleucus* were collected and only from two sites. Secondly, specimens of *E. radicatus* were collected from a single site and may represent a stunted form of the putative species. Herbarium specimens from Rocky Mountain Herbarium in Laramie suggest that *E. radicatus* and *E. ochroleucus* var. *scribneri* are more similar than my preliminary data revealed. Examination of herbarium specimens and field specimens collected during the second collecting season should determine taxonomic status of *E. radicatus*. Thirdly, because no specimens have yet been collected of the southern alpine race of *E. grandiflorus*, the species was not included in this report. Spongberg's (1972) treatment of the southern alpine race as separate from *E. simplex* is currently being re-evaluated (Hartman, Rocky Mountain Herbarium, personal communication). Synonomy of the southern alpine race of *E. grandiflorus* with *E. simplex* may not confound my studies of *E. lackschewitzii*, since this may only mean that the southern alpine race is more closely related to *E. simplex* than it is to the species of concern in this report.

Chromosome counts were unsuccessful due to lack of pollen, which accords with the statement by Nesom and Weber (1983) that *E. lackschewitzii* may differ morphologically due to apomixis and polyploidy. The single pollen grain SEM photomicrograph from Western State College substantiates pollen abnormality. Chromosome counts will be attempted from additional flower head collections during the second collecting season in order to determine ploidy level and mode of reproduction.

In summary, based on my initial investigations, I consider the relationship to *E. ochroleucus* var. *scribneri* to be more central to taxonomy of *E. lackschewitzii* than either *E. simplex* or the southern alpine race of *E. grandiflorus*. Due to the preliminary nature of these studies, however, I cannot state unequivocally that *E. lackschewitzii* should be synonymized with *E. ochroleucus*.

Table 1. Source of DNA from *Erigeron* species. Labels shown in parentheses (e.g., EL1) refer to those shown in Figs. 2-5 and Table 3.

Species	Site	Location
lackschewitzii (EL1)	Our Lake 8400'	Bob Marshall Wilderness, Lewis & Clark Natl. Forest
lackschewitzii (EL14)	Mt. Wright 7500'	Bob Marshall Wilderness, Lewis & Clark Natl. Forest
lackschewitzii (EL10)	Rocky Mtn 8380'	Bob Marshall Wilderness, Lewis & Clark Natl. Forest
radicatus (ER10)	Rocky Mtn 8380'	Bob Marshall Wilderness, Lewis & Clark Natl. Forest
ochroleucus (EO14)	Mt. Wright 7500'	Bob Marshall Wilderness, Lewis & Clark Natl. Forest
simplex (ES21)	Sacajawea Pk. 9500'	Bridger Range, Gallatin Natl. Forest
simplex (ES13)	Mt. Patrick Gass 7400'	Bob Marshall Wilderness, Lewis & Clark Natl. Forest
speciosus (ESp)	Bridger Bowl 8000'	Bridger Range, Gallatin Natl. Forest
caespitosus (EC)	Sourdough Ridge 5000'	City of Bozeman

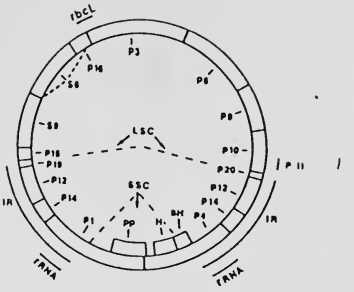


Figure 1. Locations of chloroplast DNA clones used against *Erigeron* species DNAs. From Sytsma and Gottlieb, 1986.

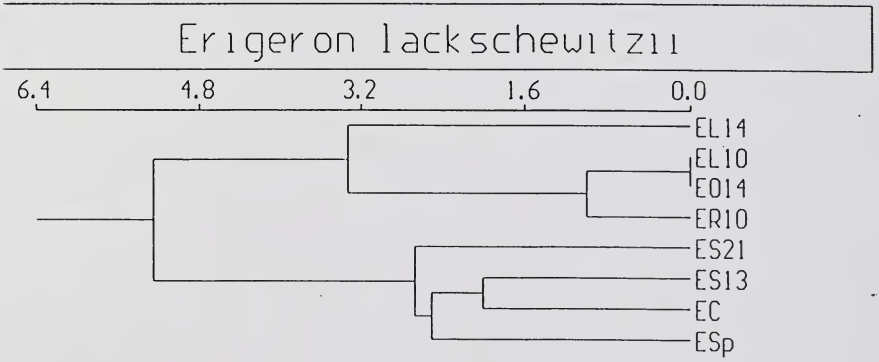


Figure 2. Dendrogram reflecting distances derived from the presence of shared cpDNA restriction sites (Table 3). Label identification: EL = *E. lackschewitzii*; EO = *E. ochroleucus*; ER = *E. radicatus*; ES = *E. simplex*; EC = *E. caespitosus*; ESp = *E. speciosus*.

Table 2. Morphological characters of *Erigeron* species utilized for multivariate statistical analysis.

Quantitative characters:

- Perennating organ length and width 5 mm below ground level
- Caudex branches -- total number, no. of perpendicular branches, and no. of decumbent branches
- Flowering stems -- number, height of tallest, and width 5 mm below involucre
- Number of flowering heads/stem
- Number of cauline leaves
- Basal leaves -- length and width of longest leaf and length and width of widest leaf
- Involucre width and height
- Outermost phyllary length and width
- Number of phyllary rows
- Ray florets -- ligule length and width, number of florets, and number of notches per ligule apex
- Disk florets -- corolla length and width
- Disk floret achenes -- number of pappus bristles, pappus height, length and width, number of ribs

Qualitative characters:

- Taproot -- presence/absence
- Flowering stems -- decumbent, ascending, or both,
  - bases red/purple or not,
  - presence of villous trichomes,
  - presence of strigose trichomes,
  - presence of glandular trichomes
- Cauline leaves -- shape same as basal, more linear than basal, spatulate, or lanceolate
  - margins undulate or not
  - extend less than or greater than halfway up stem
  - presence of strigose trichomes
  - presence of villous trichomes
  - presence of glandular trichomes
- Basal leaf shape -- presence of linear leaves
  - presence of narrowly oblanceolate leaves
  - presence of oblanceolate leaves
  - presence of spatulate leaves
- Involucre indumentum -- presence of woolly appearance
  - presence of villous trichomes
  - presence of strigose trichomes
  - presence of glandular trichomes
- Phyllaries recurved or not
- Ray floret ligule color when dried -- blue/purple
  - pink
  - white
  - same fresh as dried or not
- Ray floret ligules held obliquely to involucre or more right-angled

Table 3. Restriction site mutations of cpDNA and rDNA in six species of *Erigeron*. The ingroup and unique sequences are listed first under "Mutation." See Table 1 for label identification.

Restriction Endonuclease	Region	Mutation (kb)	Comments
cpDNA:			
*Bcl*I	S6	9.2 -- 5.2 + 4.0	
*Bcl*I	S8	5.8 + 1.6 -- 7.4	Unique to ER10
*Dra*I	S6	11.0 -- 8.8 + 2.2	
*Dra*I	S6	7.0 + 4.0 -- 11.0	Unique to EL14
*Dra*I	P3	15.7 + 8.1 -- 23.8	Unique to EL14
*Dra*I	P3	23.8 -- 14.0 + 9.8	
*Dra*I	P3	6.7 + 1.4 -- 8.1	Unique to ES21
*Dra*I	P8	1.65 + 1.65 -- 3.3	Unique to ESp
*Stu*I	S6	19.6 -- 9.8 + 9.8	
*Stu*I	S6	3.3 + 1.6 -- 4.9	Unique to EC
*Stu*I	S8	22.3 -- 12.5 + 9.8	
*Stu*I	P3	8.6 + 4.8 -- 13.4	
*Xho*I	P3	21.2 -- 17.0 + 4.2	
*Xho*I	P8	8.4 + 6.2 -- 14.6	Unique to ESp, EC
rDNA:			
*Bcl*I	rDNA	7.8 + 7.8 -- 15.6	Unique to ER10, ES13, ESp, EC
*Dra*I	rDNA	3.4 + 7.2 -- 10.6	Unique to ES13

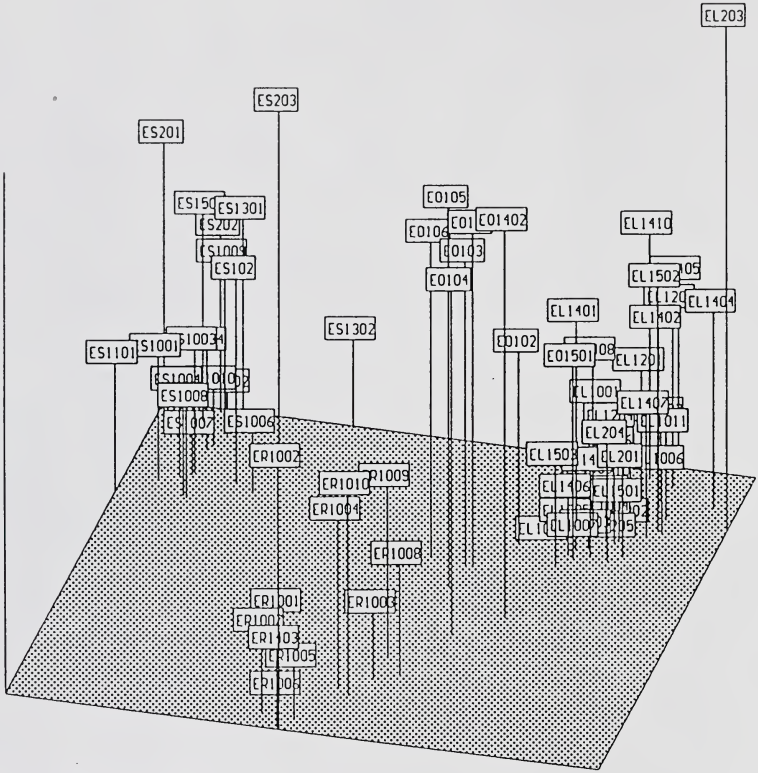


Figure 3. Ordination of phenetic distance data from four species of *Erigeron*. (See Fig. 2 for label identification.)

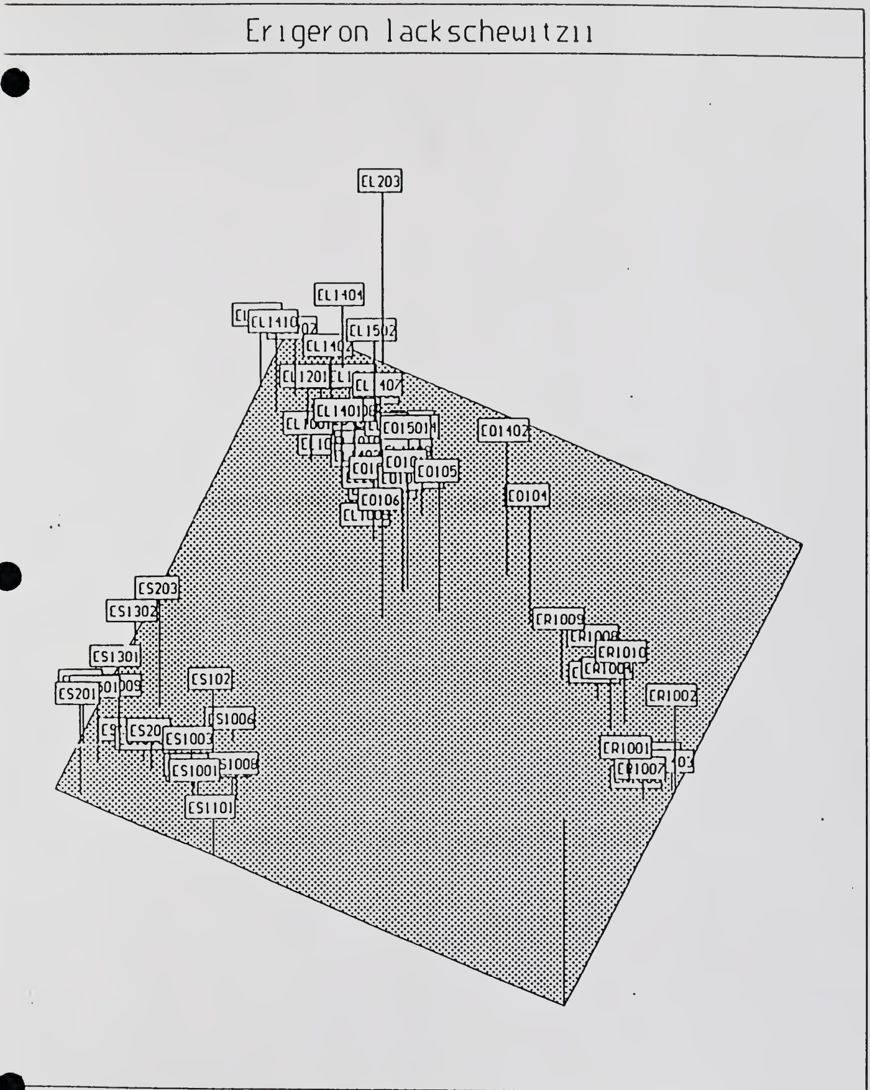
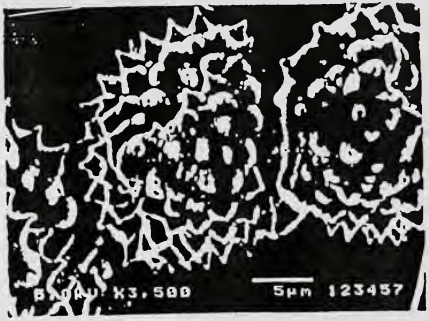


Figure 4. A different view of the phenetic distance data ordination found in Fig. 3 emphasizing distinct clustering of *E. simplex*. (See Fig. 2 for label identification.)

Figure 5. Cluster analysis of phenetic distance of all pairwise combinations of four species of *Erigeron*. (See Fig. 2 for label identification.)

6.



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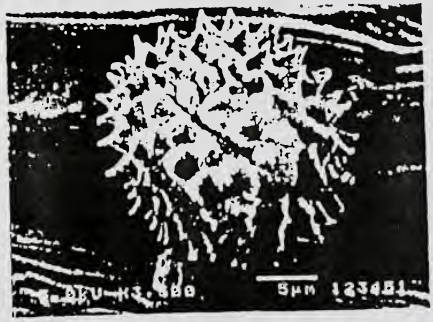

8.

Figures 6-8. Scanning electron micrographs of pollen grains from three species of *Erigeron*. 6. *Erigeron lackschewitzii*, x 3500. 7. *Erigeron simplex*, x 3500. 8. *Erigeron ochroleucus* var. *scribneri*, x 3500. SEM photos by Wayne Warnken, Western State college.

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